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ON THE BIOECOLOGY OF THE FUNGUS *PLASMOPARA VITICOLA* BERLESE & DE TONI CAUSING VINE MILDEW

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ABSTRACT

New data on the bioecological characteristics of *Plasmopara viticola* Berl. & de Toni are presented. The results of microscopic analysis of mycelium wintering of mildew causing *Plasmopara viticola* on branches and fruit bearing buds are discussed.

KEY WORDS: Vine mildew, *Plasmopara viticola* Berl. & de Toni, mycelium wintering, *Müller Curve*

Among the main diseases of vine, mildew is notable by its harmfulness. The effective control of this disease depends primarily on study of the bioecology of the disease pathogen, its wintering mechanism, and resumption of infections. Though the vine mildew has been studied for a long time, not all the bioecologic aspects of the fungus are clear at present and some key issues of the fungus causing the mildew, as well as the methods of defense against it still require significant study.

Results are presented of an investigation on the bioecology of the fungus causing vine mildew, and methods of defense against the disease. This work has been carried out during the past two decades in geographically separate areas of the Republic of Georgia (Chrelashvili 1978, 1984, 1985, 1988; Chrelashvili & Salukvadze 1985). The four geographically separate zones selected were: Kvarely, Sagaredjo (east Georgia), Maiakovsky and Gudauta (west Georgia). The results are likely applicable in other countries where viticulture is undertaken.

Past workers have agreed that one of the key issues of the bioecology of the fungus is the survival of the fungus during the period of overwintering and renewal of the fungal infection upon revegetation. It has been accepted that the mechanism of overwintering is through oospores found in the so called necrotic spots of leaves that fall into the soil, and that re-infection is accomplished by splashing of fungal spores from the soil to the leaves of the vines. The disease becomes apparent immediately after the vines produce leaves, when the day and night temperatures become 12-15° C, and rainfall occurs. Viala (1887, 1893), Speshnev (1906), Andreev (1925), Gregory (1912, 1914), Boubals (1977), Prince (1962), Verderevski & Voitovich (1970), Natsarashvili (1972), and others have all considered the problem of overwintering and renewal of infection of vine mildew.

Other authors (Yachevski 1909; Istvanp & Palinkas 1913) have expressed the opinion that presence of the fungus in wintering buds of the vine is the mechanism for overwintering and source of infection in the following growing season. Yachevski (1909) hypothesized that the infection spreads from the buds by diffusion. With the exception of Naidenova (1974), this mechanism of overwintering and re-infection has not been examined.

The necessity of a more critical determination of the exact mechanism of overwintering and re-infection of vine mildew was caused by the following facts, each of which will be discussed below:

1. Observations have indicated that the actual appearance of vine mildew is delayed by a month or more after the date predicted using the scenario implied in the accepted method of overwintering and infection.

2. The method by which the fungus spreads once infection has occurred is not known.

3. Infections were noted to spread much faster than predicted by the widely accepted method of infection.

4. Infections occurred even when the possibility of a soil borne infection source was eliminated.

1. The theoretical date of the first appearance of vine mildew is usually predicted by a curve of incubation periods (*i.e.*, the *Müller Curve* [Müller & Rabanus 1923]). Based on the determination of this date, antifungal treatments are begun. Observations during the past two decades have shown that the actual first appearance of the mildew is delayed one month and sometimes more beyond the date predicted by the *Müller Curve*.

The results of these observations from one climatic zone (Kvarely region in east Georgia) are shown in Table 1. The first column in the table shows theoretical dates of appearance, the second column shows actual dates of appearance, and the third column indicates the year in which the observations were made.

Table 1. Comparison of theoretical and actual first dates of appearance of vine mildew.

theoretical, according to the *Müller Curve*	actual	Year of observation
month/day		
05.04	06.02	1971
04.18	05.20	1972
05.05	06.05	1973
05.11	06.06	1974
04.25	05.26	1975
05.07	05.30	1976
04.28	05.31	1977
05.10	06.05	1978
05.29	07.02	1979
05.14	06.18	1980
04.27	05.17	1981
05.15	07.02	1982
05.25	07.05	1983
05.16	07.02	1984
05.15	06.18	1985
05.10	06.25	1986

The observations made regularly from 1971-1986, have shown that the appearance of vine mildew on the plants coincides not with the predicted appearance based on climatic variables, but with the opening of floral buds. In each case, mildew was first observed as, or shortly after flower buds opened. Widely accepted theory predicts that infection will occur earlier, when leaf buds break. However, as we have observed, the appearance of the disease is correlated with a specific phenological phase of vine, namely with the "preflowering" period. At this stage the plant mobilizes large amounts of its resources to support the flowering and fruiting, and is richest from the viewpoint of nutrient medium. The combination of the availability of these resources, along with favorable climatic conditions, formates most favorable conditions for rapid development of the fungus.

2. Following the widely accepted mechanism of infection and incubation period for the fungus, the process of continuous formation of the mildew on vine leaves is not satisfactorily explained. Raikov & Ionov (1958), Dudin & Dementieva (1958), and others have suggested that night dew is responsible for the continuous formation of vine mildew.

However, observations reported here, taken over several years, show that the process of continuous formation of vine mildew takes place even without night dew or rain. Table 2 contains the meteorological data from July 1976 at the experimental site, along with data on vine mildew infection. During the two week observation period, newly infected leaves were observed each day, even though neither rain nor night dew occurred during this period. These observations are particularly interesting in light of the fact that the fungus is known to infect by entering through the stoma of leaves when water is present.

3. An inconsistency was noted between leaf age and incubation periods for appearance of vine mildew as predicted by use of the *Müller Curve*. In particular, when incubation period according to the *Müller Curve* was 5-6 days, fungal damage was also observed on 2-3 day old leaves. According to previously accepted patterns, infection should take place through the edges of the leaves when water is present and the infestation should become observable after an incubation of 5-6 days. The fact that infections appear in 2-3 day old leaves indicates that if the incubation actually takes 5-6 days, then infection could not have occurred as described. Consequently, an internal infection source is indicated by these data. Figure 1 shows a curve depicting percentage of damaged leaves by age of leaves. The abscissa corresponds to the age of leaves (in days), and the ordinate corresponds to the percentage of damaged leaves. Maximum damage appears in 8-9 day old leaves, with minimum damage in 2-3 day old leaves.

4. According to the literature, grafts and seedlings are most susceptible to mildew, and infection originates from soil as a result of raindrops splashing contaminated soil onto the leaves or by wind carrying oospores from contaminated soil onto the leaves. Experiments were conducted to test the hypothesis

Table 2. Climatic data and observations of leaves during July 1976. The total number of leaves observed was 890. No precipitation nor dew was recorded during the period.

date	air temperature ° C		relative humidity %		ground temperature ° C		dew point ° C		number of infected leaves
	3am	6am	3am	6am	3am	6am	3am	6am	
07.01	19.7	19.2	70	72	17	19	14	14	5
07.02	17.8	18.1	82	79	16	19	16	19	6
07.03	17.5	17.8	64	67	15	17	11	12	10
07.04	15.8	16.4	53	52	13	15	6	6	12
07.05	17.8	14.5	81	75	11	14	10	10	6
07.06	15.2	16.2	76	75	13	16	11	12	7
07.07	16.4	18.2	78	73	15	18	13	13	4
07.08	19.0	19.2	64	63	17	20	12	12	11
07.09	18.5	18.3	68	76	18	19	12	14	12
07.10	19.5	19.7	77	79	18	21	15	16	4
07.11	21.2	21.2	77	71	19	20	16	16	6
07.12	19.8	20.1	76	78	19	21	15	16	2
07.13	18.0	18.4	81	78	14	17	15	15	1
07.14	19.9	17.3	64	71	18	18	13	12	1

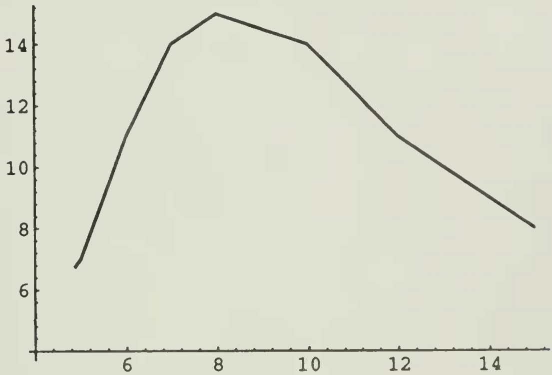

Fig 1. The amount of damaged leaves in percentage (the axis of the ordinate) vs the age of the leaves in days.

of soil originated infection.

In order to test the accepted methods for infection, 80 square meters of prepared soil was covered with polyethylene. Holes were made in the polyethylene just large enough to plant the seedlings. A control plot was made with plants placed in uncovered soil.

Experimental plants grew more rapidly in general, and expressed mildew infections approximately 20 days earlier than control plants. The more rapid growth was likely due to elevated temperature and moisture levels under the polyethylene. However, since the possibility of infection by splashing of contaminated soil had been excluded, the more rapid appearance of the fungal infections on experimental plants can only be explained if the infection were already present in the seedlings, in which case, the more rapid appearance of mildew on experimental plants could also be explained by the elevated temperature and moisture levels under the polyethylene.

Another experiment in which the experimental treatment involved seedlings placed in closed pots in a greenhouse showed similar results (*i.e.*, mildew infections became apparent approximately 20 days earlier in experimental plants than in control plants). In this case, the possibility of wind borne infection, as well as water borne infection had been excluded. These data suggest that the source of these outbreaks of mildew is from within the tissues of the plant, and that the appearance of the fungus is not predicated on an infection source,

Table 3. The results of light microscopic analysis.

Sample	Number of sections	Number of sections where mycelium was observed
1	10	1
2	10	0
3	10	2
4	10	1
5	10	3

but merely the presence of conditions under which the fungus can grow rapidly and become apparent.

The possibility of an infection source from within the plant tissue is suggested by the well known fact that vine mildew was introduced to Europe by a sample phylloxeraproff graft brought from America in 1887. It is also known that other perenosporals (such as *Plasmopara*) winter in the plants as a latent infection. It would appear based on the data presented here, that the vine mildew is no exception.

The data presented above lead to the conclusion that previously accepted hypotheses on the source of infection of vine mildew are inaccurate, and the inappropriateness of use of the *Müller Curve* to predict when vine mildew becomes apparent. Further, the data suggest that the source of early season vine mildew outbreaks is a latent infection within the plants. If this is the case, then the fungal infection would be expected to be present in samples of the plant tissue. Microscopic analyses were conducted to determine whether the fungus was present in apparently uninfected plant tissue.

Sections were made on a microtome from shoots collected in the spring, that had expressed infection during the previous growing season. Light microscope examination showed that fungal mycelium was present in many sections (Table 3). The fact that mycelium was not observed in all sections suggests that the fungus may not be present in all tissues. However, even though not found throughout the plant, the presence of fungal mycelium in any portion of the plant would allow much more rapid expression of the fungus than if the infection were required to be introduced from outside the plant.

Electron microscope analyses carried out using a YEM-10013 transmission electron microscope, provided further information on the existence of fungal infections in dormant plant tissue. Experimental material was treated to prevent other diseases than vine mildew. Control material was free of all known pathogens. Longitudinal sections were made and observed under the microscope. Cells of control tissues had well defined edges, quite thick osmophilic globulations, and roundish mitochondria. In experimental tissue, cell boundaries were ill defined and the fungal mycelium was clearly seen.

CONCLUSION

It is clear that previously accepted hypotheses considering the infection and spread of vine mildew are inaccurate, and that treatment protocols based on those hypotheses are flawed. Specifically, early season treatments to control the spread of the disease, or prophylactic treatments to prevent infection are unnecessary. Based on the findings of this study, new treatment protocols have been developed for use in Georgia. These treatments have provided control as well as previous treatments, but since they are made less often, a substantial savings in treatment expenditures has been realized.

The currently used treatment schedule is as follows:

1. A sanitary treatment or "autumn measure", carried out as soon as the vintage is completed. This is a systemic treatment aimed at reducing the amount of fungal material available for overwintering while the fungus is still localized in the plant.

2. A preblooming treatment directed at reducing spread of any fungus in the plant and timed to coincide with the first outbreak of the fungus without treatment.

3. Immediately after flowering, directed at reducing spread of any fungus in the plant.

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